BREVIORA

Museum of Comparative Zoology

US ISSN 0006-9698

CAMBRIDGE, MASS. 5 JANUARY 2021 NUMBER 569

OSTEOLOGY OF *PARABROTULA PLAGIOPHTHALMUS* (OPHIDIIFORMES: BYTHITOIDEI: BYTHITIDAE)

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ABSTRACT. *Parabrotula* (Zugmayer 1911a), included in the family Bythitidae (Ophidiiformes), is a genus of small viviparous mesopelagic fishes with two valid species. *Parabrotula plagiophthalmus* is known from collections made worldwide except in the eastern Indian, eastern Pacific, and western South Atlantic Oceans; most specimens are from the North Atlantic Ocean. Most anatomical data for *P. plagiophthalmus* are limited to aspects of external anatomy, the reproductive system, and meristic data that can be gathered through radiographs; virtually no data on other aspects of the osteology of *P. plagiophthalmus* are available. In this study, we describe the skeleton of *P. plagiophthalmus* from cleared and stained specimens and computed tomography data. Many aspects of the skeleton of *P. plagiophthalmus* are reduced (subopercle; ceratobranchial 5; gill rakers; gill filaments; distal radials of median pterygiophores; caudal skeleton; posttemporal; supracleithrum; scapula; distal pectoral radials; pelvic girdle and fins; scales) or absent (mesethmoid; nasal; pterosphenoid; intercalar; infraorbital bones; endopterygoid; coronomeckelian; supramaxilla; pharyngeal toothplates). All bones of the skull are extremely thin dermal or perichondral ossifications, with little to no endochondral components. The vomer supports a variable number of teeth (0–3). The pars autopalatina and its ossification are separated from the pars metapterygoidea and pars quadrata and their ossifications; the thin ectopterygoid bridges these elements of the suspensorium. The lower jaw is very large compared with all other elements of the viscerocranium. The pectoral radial plate was never observed to be divided into separate radial elements. We discuss the skeleton of *Parabrotula* in the context of other taxa but call attention to the need for further comparative studies of the anatomy of ophidiiform fishes.

KEY WORDS: Parabrotulidae; mesopelagic fishes; skeletal anatomy; cleared and stained specimens; computed tomography

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INTRODUCTION

The family Bythitidae is a species-rich member of the order Ophidiiformes, with at least 32 genera and 116 species (Møller et al., 2016). These fishes are commonly known as the viviparous brotulas, reflecting their reproductive biology, and are diagnosed by

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Figure 1. *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Illustration from Zugmayer (1911b, plate 6, fig. 5). B, Photograph of whole specimen in left lateral view, anterior to left (MCZ 56866, 34.1 mm SL).

details of the male copulatory organ (having the penis-like structure continuous with a fleshy hood anterior to the anal fin origin and the absence of pseudoclaspers), as well as combinations of meristic characters (Møller et al., 2016), although there is substantial intrafamilial variation in the meristic characters and large overlap with the Dinematichthyidae, the sister group of Bythitidae. The taxonomic concept of Bythitidae proposed by Møller et al. (2016), which was based on analysis of mitochondrial and nuclear gene loci, included the formerly recognized groups of ophidiiform fishes in Bythinae, Brosmophycinae, and Aphyonidae. Campbell et al. (2017), in a more taxonomically restricted molecular data set, also found the enigmatic family Parabrotulidae to be nested deeply within the newly constituted Bythitidae. Inclusion of taxa included in both of these families in an expanded Bythitidae was accepted by Betancur-R et al. (2017).

Parabrotula (Zugmayer 1911a) is a genus of small viviparous mesopelagic teleostean fishes with two valid species (Fricke et al. 2019). *Parabrotula plagiophthalmus* Zugmayer 1911a (see Zugmayer 1911b for a fuller description and illustration of the specimen; Fig. 1A) was described on the basis of a single specimen collected in the northeastern Atlantic Ocean on 9 August 1909 aboard the SS *Princesse Alice* (44°04′N, 19°42′W, station 2870, 0–1,500 m). Beebe (1932) described a second species, *Parabrotula dentiens*, on the basis of a single small specimen collected southeast of Bermuda; Nielsen (1968) concluded that this taxon is a junior synonym of *P. plagiophthalmus*. Miya and Nielsen (1991) described *Parabrotula tanseimaru* on the basis of 25 specimens collected in Sagami Bay along the southern coast of Japan. *Parabrotula tanseimaru* differs from *P. plagiophthalmus* in number of dorsal-fin rays and vertebrae, as well as position of the dorsal fin insertion. *Parabrotula plagiophthalmus* is known from collections made worldwide (Nielsen et al., 1990, fig. 4; also including the western Indian Ocean from the coast of Somalia, Museum of Comparative Zoology, Harvard [MCZ], 130654 and MCZ 143362), with the exception of the eastern Indian, eastern Pacific, and western South Atlantic Oceans, as well as the polar Arctic and Southern Oceans. As noted by Nielsen et al. (1990), however, most specimens (89% included in their study) are from the North Atlantic Ocean.

Zugmayer (1911a, b) initially described the genus *Parabrotula* as belonging to the family Brotulidae because of the presence of free branchial membranes, although he did note that the genus shared the absence of pelvic fins with Zoarcidae (he also acknowledged the absence of these fins in other forms thought to share an affinity with brotulids, such as *Barathronus* and *Aphyonus* spp.). In his redescription of the genera *Parabrotula* and *Leucobrotula*, a monotypic and rarely collected genus from the northeast Atlantic, Nielsen (1968) concluded that the two genera showed greater similarity to the Zoarcidae, particularly with regard to the one-to-one correspondence of dorsal and anal fin rays to vertebrae (compared with more than one dorsal and anal fin rays per vertebra in ophidioids). Furthermore, he suggested that

these two genera are most closely related to each other and established the new subfamily Parabrotulinae within Zoarcidae (the subfamily name was used at the family level, Parabrotulidae, by Nielsen et al. 1990). Recently, Campbell et al. (2017) studied the phylogenetic affinities of Parabrotulidae with genetic data and concluded that the family (represented only by *P. tanseimaru*) was deeply nested within the ophidiiform family Bythidae.

Most anatomical data for *P. plagiophthalmus* are limited to aspects of external anatomy (Fig. 1), the reproductive system, and meristic data that can be gathered through radiographs. Virtually no data on other aspects of the osteology of *Parabrotula* or *Leucobrotula* are available. The comparative osteology of Ophidiiformes (i.e., Ophidioidei + Bythitoidei) generally is not well known, and this has hampered anatomically based systematic studies of these fishes (see Carnevale and Johnson, 2015, for a recent review and summary of the skeleton of both ophidiiform lineages). Given the opportunity to clear and stain a large specimen of *P. plagiophthalmus* (Virginia Institute of Marine Science [VIMS] 25002), which stained particularly well, here we describe its skeleton from cleared and stained specimens and computed tomography data. These data are intended to serve as a contribution to the anatomy of the family and of Ophidiiformes generally, although detailed comparisons and a phylogenetic assessment of the position of *P. plagiophthalmus* is beyond the scope of the present study.

MATERIALS AND METHODS

Specimen preparation

Three specimens of *P. plagiophthalmus* were cleared and stained following protocols based on Dingerkus and Uhler (1977). Radiographs of whole, alcohol-preserved specimens were used to collect morphometric and meristic data; although these types of data were not emphasized in this study, they are available from Nielsen (1968) and Nielsen et al. (1990), for which this study serves as a complement.

Specimens were examined with dissecting microscopes. Images were captured as Z-stacked images with a Zeiss HD Axiocam camera attached to a Zeiss Discovery V20 stereomicroscope with AxioVision software. Color balance and sharpness were adjusted by Adobe Photoshop CC. Line drawings were rendered by Adobe Illustrator CC software from photomicrographs.

Specimen scans were performed with a SkyScan 1173 micro-CT at 37 kV and 190 μA and a voxel size of 7.1 μm. Scans were reconstructed by NRecon software, optimizing settings on the basis of scan results. Slices were filtered in Bruker DataViewer software to maximize bone contrast and then exported at original resolution. Resultant slices were rendered in Bruker CTVox software by the maximum intensity projection method.

Specimens examined

Specimens are indicated as cleared and stained (c&s) or alcohol-stored (a) specimens. Standard lengths (SLs) are given. Institutional abbreviations follow Sabaj (2019).

Bythitidae

Parabrotula plagiophthalmus. MCZ 56867 (21.0 mm SL, a); MCZ 14955 (29.5 mm SL, c&s); MCZ 143362 (30.1 mm SL, a); MCZ 161535 (30.4 mm SL, a); MCZ 85403 (30.5 mm SL, a); MCZ 62266 (33.9 mm SL, a); MCZ 56866 (34.1 mm SL, a); MCZ 56859 (35.0 mm SL, a); MCZ 97338 (36.9 mm SL, a); MCZ 130654 (37.5 mm SL, a); MCZ 56870 (37.5 mm SL, a); MCZ 149550 (39.2

mm SL, a); MCZ 62267 (41.6 mm SL, c&s); MCZ 62265 (42.2 mm SL, a; computed tomography [CT]-scanned); MCZ 62268 (42.2 mm SL, a); MCZ 149551 (2 specimens; not measured, a); MCZ 101694 (specimen is c. 30 mm, but missing caudal fin and posterior vertebrae, a); VIMS 25272 (23.5 mm SL, a); VIMS 25002 (47.3 mm SL, c&s; female with embryos in uteri).

Other Ophidiiformes

Brosmophycis marginata: OS 3855 (1 a, 235 mm SL).

Dinematichthys iluocoeteoides: UW 25889 (1 c&s, 50.1 mm SL).

ANATOMICAL DESCRIPTIONS

Skull roof and neurocranium

All bones of the neurocranium and skull roof are extremely thin dermal or perichondral ossifications with little to no endochondral components (exceptions are noted below) and the underlying cartilages of the neurocranium showing through on cleared and stained specimens (Figs. 2A–C). As such, the margins of bones are difficult to determine but could be examined by changing lighting conditions, focus plane, and angle of the specimens and confirmed in the CT scans (Figs. 2D–G). Several bones of the skull roof and neurocranium were determined to be absent, including the mesethmoid, nasals, pterosphenoids, and intercalars. (Bones that are absent in other anatomical systems are noted in those sections.)

In dorsal view, the neurocranium and skull roof have a narrow anterior region and a broad, rounded otic region. The left and right lateral ethmoids appear to be continuous across the midline. Whether this results from fusion with a median mesethmoid or a loss of the mesethmoid and subsequent fusion of the left and right lateral ethmoids is unknown. The frontals are the largest elements of the skull roof, extending over half the length of the neurocranium. Anteriorly, they taper gently to a point just over the posterior margin of the lateral ethmoid. On its ventral surface, the frontal has a descending lamina in the anterior portion of the orbit (Fig. 3B). The broad posterior margins of the frontals meet the similarly wide anterior margin of the parietals. The parietals are roughly triangular in shape, with the posterior point extending posteriorly to reach between the widely spaced exoccipitals. The lateral margin of the posterior region of the neurocranium is bordered by the autosphenotic, pterotic, and exoccipital; the epioccipital is positioned between the exoccipital and the parietal (Fig. 3A). The autosphenotic reaches anteriorly to the midorbital region, where it is continuous with the cartilage of the neurocranium. Posteriorly, on its ventrolateral surface, the autosphenotic forms, together with the prootic, the anterior portion of the facet for the hyomandibula (Figs. 3B, C). The pterotic is large and extends dorsally to meet the parietal (Figs. 3A, B), and ventrolaterally to be intercalated between the prootic and exoccipital in ventral view (Fig. 3C). Ventrally, it supports the posterior facet for articulation of the hyomandibula (Fig. 3C). The prootic is a largely rounded bone that forms the posterior margin of the orbit and bears a distinct notch in its anterior margin, which marks the exit of the trigeminal nerve (i.e., the foramen for this nerve is not completely enclosed in the prootic). A foramen is positioned posteriorly on the left prootic of the specimen illustrated in Figure 3C, but it is unclear whether it is a separate exit for a nerve or a blood vessel.

The occipital region is formed by the epioccipitals, exoccipitals and basioccipital. Because they are exceptionally thin, the

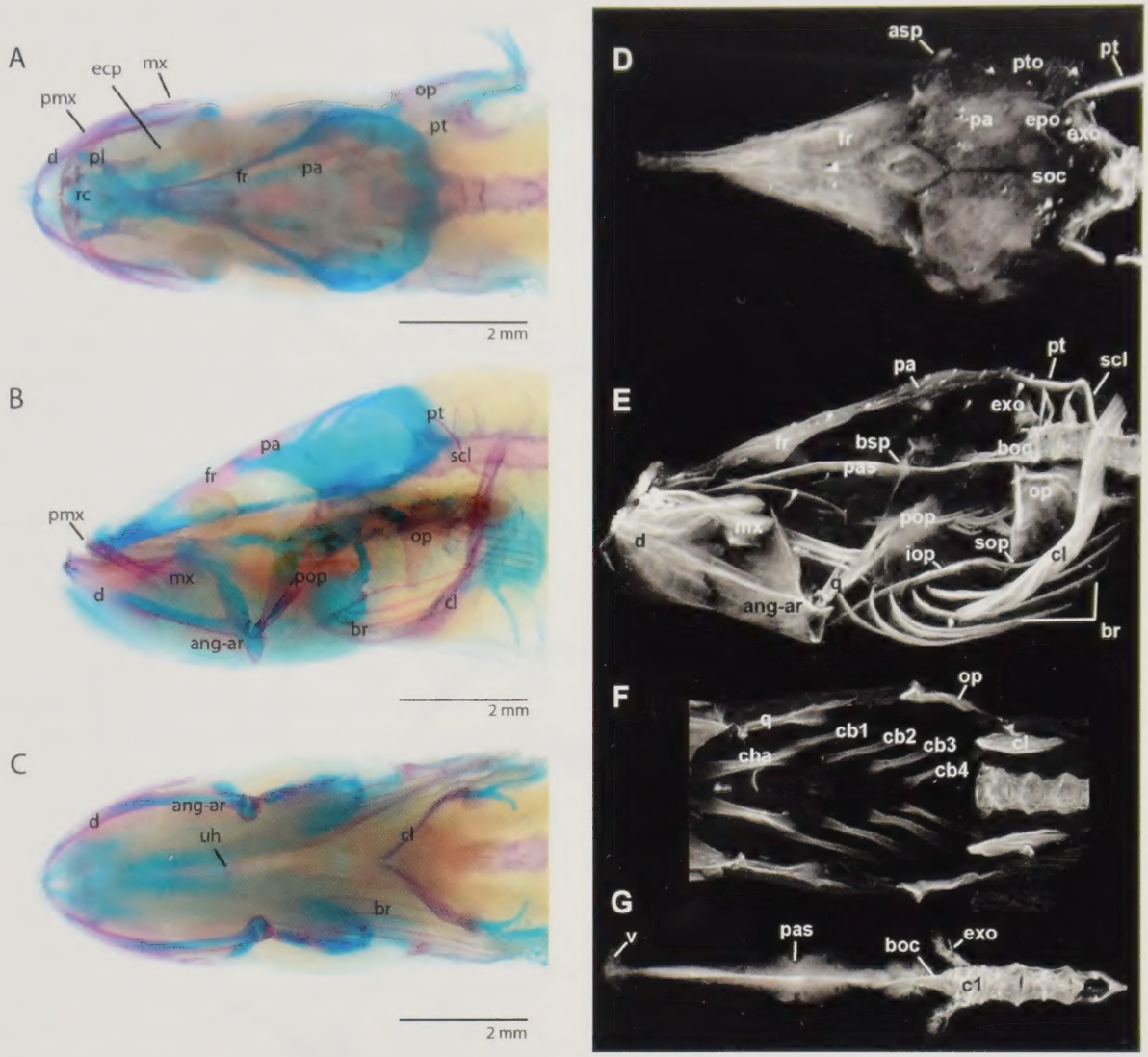

Figure 2. Skull of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Dorsal, B, lateral, and C, ventral views of cleared and stained specimen. VIMS 25002 (44.9 mm SL). D–G, CT-scanned specimen (MCZ 62265, 42.2 mm SL): D, dorsal view of neurocranium and skull roof; E, lateral view of skull; F, dorsal view showing arrangement of ceratobranchials in situ; G, isolated ventral portion of the braincase and anterior vertebral column in ventral view. Note that the position of the sensory canal pores are visible in D and E as white spots (unlabeled). Abbreviations: ang-ar, anguloarticular; asp, autosphenoid; boc, basioccipital; br, branchiostegals; bsp, basisphenoid; c1, centrum 1; cb, certatobranchial; cha, anterior ceratohyal; cl, cleithrum; d, dentary; ecp, ectopterygoid; epo, epioccipital; exo, exoccipital; fr, frontal; iop, interopercle; mx, maxilla; op, opercle; pa, parietal; pas, parasphenoid; pl, palatine; pmx, premaxilla; pop, preopercle; pt, posttemporal; pto, pterotic; q, quadrate; rc, rostral cartilage; scl, supracleithrum; soc, supraoccipital; sop, subopercle; uh, urohyal; v, vomer.

margins of the epioccipitals are difficult to trace, but they appear to be small, rounded bones that are intercalated between the parietals, pterotics, and exoccipitals; they were only detected because of their association with the posttemporal. The exoccipitals extend dorsally, matching the posterior margin of the pterotic (Figs. 3A, B) but do not meet in the midline; the otic region is rather defined by a large cartilaginous region

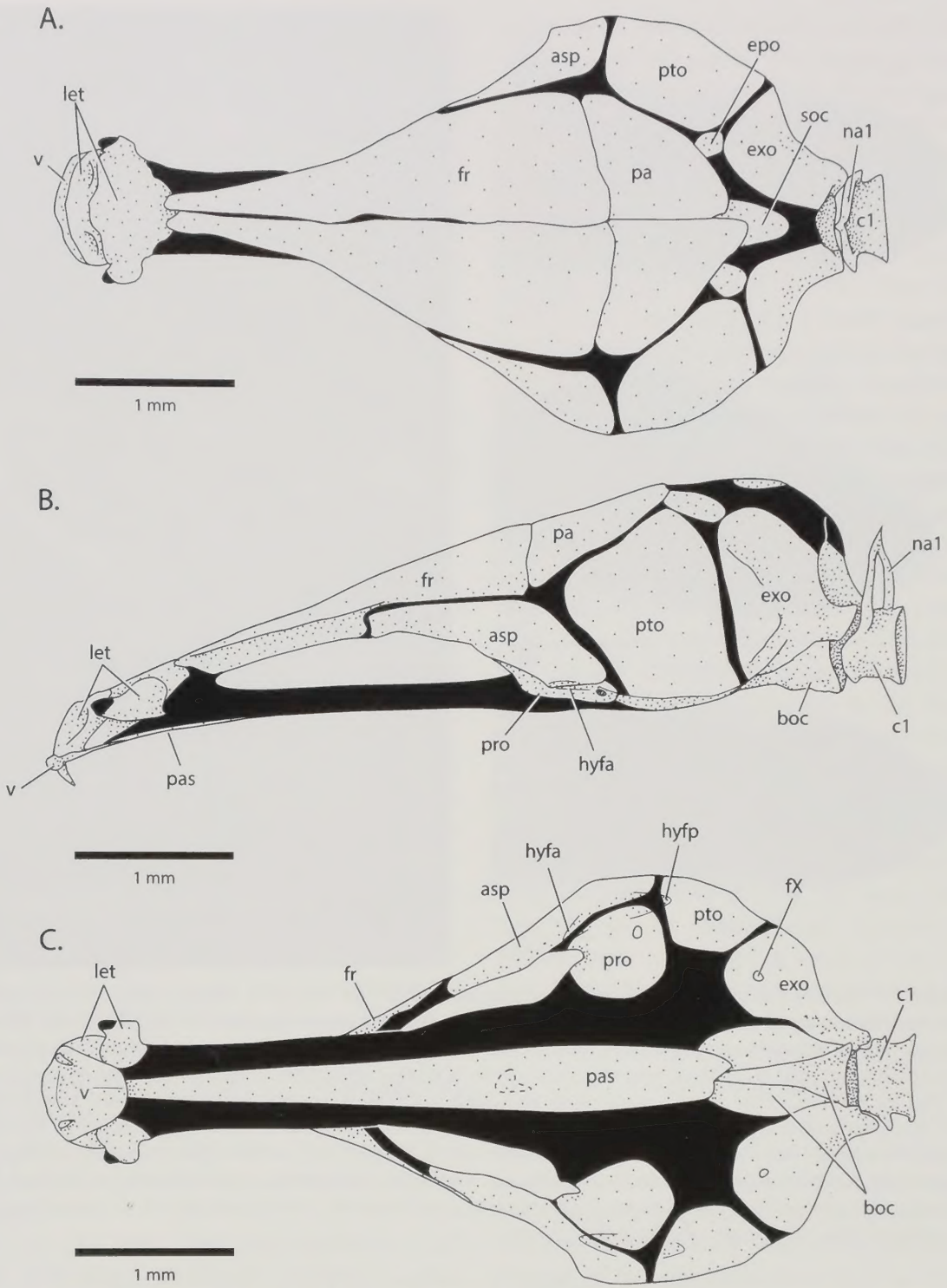

Figure 3. Neurocranium of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Dorsal, B, lateral, and C, ventral views. VIMS 25002 (44.9 mm SL). Abbreviations: asp, autosphenotic; boc, basioccipital; c1, centrum 1; epo, epioccipital; exo, exoccipital; fr, frontal; fX, foramen for the vagal nerve. hyfa, anterior portion of the fossa for the head of the hyomandibula; hyfp, posterior portion of the fossa for the head of the hyomandibula; let, lateral ethmoid; na1, neural arch 1; pa, parietal; pas, parasphenoid; pro, prootic; pto, pterotic; soc, supraoccipital; v, vomer. Note the position of the basisphenoid is indicated in panel C by a dashed line (unlabeled).

that forms the dorsal portion of the foramen magnum (Fig. 3A). The left and right exoccipitals each bear an enlarged posterior process that forms an articulation point that contacts a facet on the first vertebra (Fig. 3B). Ventral to these processes, the basioccipital also contacts the first centrum through its centrum-shaped posterior surface. The main axis of the basioccipital continues anteriorly to a point that is overlapped by the parasphenoid. Laterally, the basioccipital has broadly rounded margins that contact the exoccipitals posteriorly and the parasphenoid anteriorly (Fig. 3C). The median supraoccipital, similar to the epioccipitals, is weakly ossified and difficult to delimit. It appears to be an ovoid element that is covered by the posterior tips of the parietals and does not reach the dorsal margin of the foramen magnum.

The vomer is the anteriormost element in ventral view, forming the anterior margin of the ethmoid region (Fig. 3); this is the region where the vomer is most heavily ossified. It continues posteriorly as a broad, flat sheet of bone forming a thin veneer over the ventral surface of the ethmoid cartilage. The vomer bears a median ridge that extends posteriorly to the contact point with the anterior tip of the parasphenoid. In the specimen illustrated in Figure 3 the vomer supports two prominent large, recurved teeth that are positioned laterally along the anterior margin (Figs. 3B, C); however, the vomers of the other two cleared and stained specimens examined here are edentulous. Nielsen (1968) reports three vomerine teeth on a single specimen. The parasphenoid is narrow and elongate, extending most of the length of the neurocranium. An ascending process of the parasphenoid is absent, and posteriorly it overlaps the anterior portion of the basioccipital. The small median basisphenoid is positioned at about the midpoint of the neurocranium, although it is hidden from view (Fig. 3C).

Infraorbital bones

There are no infraorbital bones present in *P. plagiophthalmus*.

Opercular bones, suspensorium, and oral jaws

Although largely complete as series, many of the elements in these anatomical units of *P. plagiophthalmus* are small and weakly present compared with other ophidiiforms. The opercle is large and roughly oval in shape, with a flattened to slightly concave anterior margin (Fig. 4). The facet for the hyomandibula is at the anterodorsal corner of the margin of the opercle and is well developed as a distinct, cup-shaped concavity. The subopercle is a threadlike and weakly ossified element that follows the ventral margin of the opercle (Fig. 4B). It extends anteriorly beyond the margin of the opercle to reach and overlap the posterior tip of the interopercle. The interopercle is broad anteriorly but tapers irregularly posteriorly (Fig. 4) overlying the anterior half of the sliverlike subopercle (Fig. 4). The posterodorsal margin of the interopercle is relatively straight, whereas the ventral margin is distinctly rounded (Fig. 4). The preopercle (Fig. 4A) lies posterolateral to the bones of the hyosymplectic, although it barely overlaps the quadrate and symplectic. It is a roughly rectangular or oval bone with an irregularly shaped margin (Fig. 4).

The hyomandibula and the symplectic are both well ossified and are connected by a remnant of the hyosymplectic cartilage (Fig. 4). The main body of the hyomandibula is roughly square shaped and fairly broad compared with the symplectic, and has an elongate opercular head extending posteriorly, articulating with the opercle. The

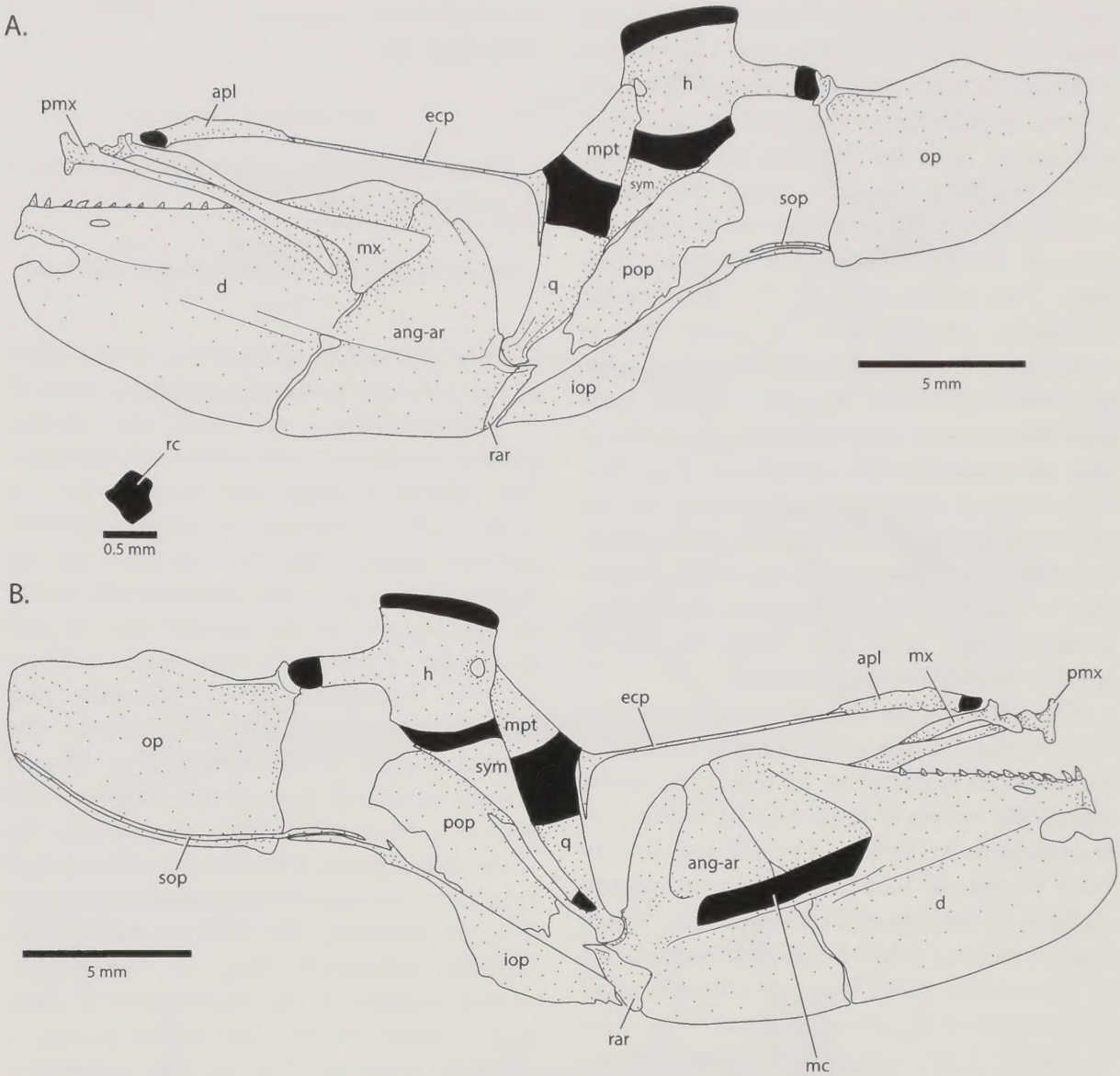

Figure 4. Opercular series, suspensorium, and oral jaws of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Lateral (inset: dorsal view of rostral cartilage) and B, medial views. VIMS 25002 (44.9 mm SL). Abbreviations: ang-ar, anguloarticular; apl, autopalatine; d, dentary; ecp, ectopterygoid; h, hyomandibula; iop, interopercle; mc, Meckel's cartilage; mpt, metapterygoid; mx, maxilla; op, opercle; pmx, premaxilla; pop, preopercle; q, quadrate; rar, retroarticular; rc, rostral cartilage; sop, subopercle; sym, symplectic.

opercular head is nearly as long as the width of the main body of the hyomandibula. The foramen for the hyomandibular trunk of the combined facial and lateral line nerve is positioned along the anterior margin of the hyomandibula at the level of the opercular process. There is only a single dorsal head of the hyomandibula for the articulation with the neurocranium. Ventral to the hyomandibula is an elongate triangular symplectic that lies against the medial surfaces of the metapterygoid and quadrate anteriorly and the preopercle posteriorly (although its contact with the preopercle is very slight).

The palatoquadrate and its ossifications include the metapterygoid, quadrate, ectopterygoid, and autopalatine; the endopterygoid is absent. The posterior portion of the suspensorium comprises the metapterygoid dorsally and the quadrate ventrally (Fig. 4). The pars metapterygoidea, and, with that, its ossification, is roughly triangular, with its apex reaching the level of the hyomandibular foramen. The quadrate, which is separated from the metapterygoid by a large block of cartilage, is relatively tall and quadrilateral in shape, with a distinct, rounded articulatory surface for the lower jaw. The posteroventral process of the quadrate is present but short (Fig. 4B). The ectopterygoid is exceptionally thin and is broadest at its far posterior tip, where it contacts the cartilaginous gap between the metapterygoid and the quadrate (Fig. 4). The anterior tip of the ectopterygoid contacts the tapered posterior point of the autopalatine. The autopalatine is well ossified and present as an anteriorly cartilage-capped tube of perichondral bone that is broadest anteriorly. Notably, the anterior and posterior chondral components of the suspensorium (i.e., the pars autopalatini anteriorly and the posterior pars metapterygoidea and pars quadrata, respectively, and their ossifications) are entirely separate from one another, with only the thin ectopterygoid connecting them.

The lower jaw is very large compared with all other elements of the viscerocranium. Meckel's cartilage is present and well developed (Fig. 4B); the coronomeckelian is absent. The articular surface that receives the quadrate is formed by the retroarticular and the articular component of the anguloarticular. The retroarticular is small and forms the entire posterior margin of the lower jaw ventral to the jaw joint in both lateral and ventral views (Fig. 4). The anguloarticular is exceptionally large and triangular. The relatively narrow coronoid process of the anguloarticular (Fig. 4B) broadly overlaps the medial surface of the more anterior parts of the anguloarticular. Taken as a whole, the anguloarticular is roughly triangular in shape and broad anteriorly, where it meets the posterior margin of the dentary, which is the largest element of the lower jaw. The anterior tip of the dentary is robust but notched. There are 12 small, medially directed teeth on both the left and right dentaries in the specimen shown in Figure 4, although we observed that the number of teeth varies among our specimens of *P. plagiophthalmus*, both between individuals as well as asymmetrically within an individual (see Nielsen, 1968, table 1).

The upper jaw consists only of the maxilla and premaxilla. There are no supramaxillae. The premaxilla is elongate and narrow (Fig. 4A), with a weakly formed ascending process that contacts the broad but flat rostral cartilage (Fig. 4A, inset). The premaxillae of the specimen illustrated in Figure 4 are edentulous, although Nielsen et al. (1990) reported that a few (five of 141 specimens examined) have teeth; this did not appear to be related to ontogeny. The maxilla is, like the premaxilla, elongate and gracile with slightly enlarged anterior processes. The posterior end of the maxilla is, conversely, flared and broad, with a ventrally directed extension (Fig. 4A).

Ventral portion of the hyoid arch

The ventral portion of the hyoid arch in *P. plagiophthalmus* is complete, with all typical elements present and ossified (Fig. 5). The interhyal is a relatively large, rod-like element with a perichondral ossification in the middle of the body. Its ventral cartilaginous tip articulates with a distinct rounded process that extends from the posterior margin of the posterior ceratohyal. This process is accentuated by a distinct notch

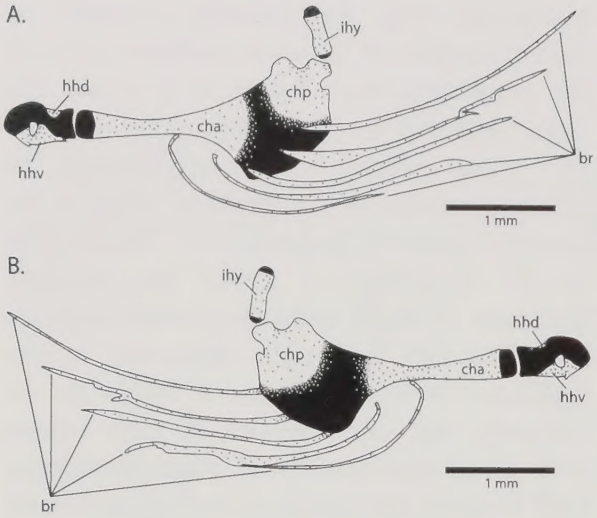

Figure 5. Ventral portion of the hyoid arch and branchiostegals of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Lateral and B, medial views. VIMS 25002 (44.9 mm SL). Abbreviations: br, branchiostegals; cha, anterior ceratohyal; chp, posterior ceratohyal; hhd, dorsal hypohyal; hhv, ventral hypohyal; ihy, interhyal.

in the posterior margin of the element. The anterior ceratohyal is elongate, with dorsal and ventral margins that are gently curved. The anterior and posterior ceratohyals are broadly separated by cartilage (Fig. 5). Similarly, the dorsal and ventral hypohyals are separated from one another by cartilage. The dorsal hypohyal is the smaller of the two hypohyal elements and is not in contact with the hypohyal foramen, whereas the ventral hypohyal is larger, and its dorsal margin forms the ventral portion of the foramen. The basihyal is described and illustrated in the next section with the ventral portion of the gill arches.

Five thin, elongate branchiostegals are present in the *P. plagiophthalmus* specimen illustrated in Figure 5, the two anteriormost of which are supported by the anterior ceratohyal; the more posterior branchiostegals are associated with the cartilage between the anterior and posterior ceratohyals (Fig. 5). Nielsen (1968) reported six to seven branchiostegals in his specimens.

Gill arches

In general, the gill arches of *P. plagiophthalmus* have the appearance of being structurally reduced (i.e., weakly ossified). In our specimens, we observed no mineralized gill rakers, although Nielsen (1968:233) reported eight to 10 "poorly developed and edentate" gill rakers on the first arch and noted that they were "better developed on the second and third than on the first arch." Gill rakers are present in our specimens (not counted), so it is possible that our specimens were decalcified, or some other artefact of preservation obscured the gill rakers because our specimens are within the same size range as those examined by Nielsen. The cartilages associated with the gill filaments are small and rudimentary. There are only approximately 10 cartilaginous gill filaments along the first arch in our largest specimen (VIMS 25002).

The dorsal gill arches comprise three pharyngobranchial (pb2–4) and four epibranchial (eb1–4) cartilages; no ossifications or tooth plates are present in the dorsal gill arches (Fig. 6). Pharyngobranchials 2 and 3 (pb2–3) are large irregularly rectangular cartilages oriented parallel to the long axis of the skull; pharyngobranchial 4 (pb4) is smaller and is oriented obliquely (Fig. 6). The four epibranchials are all approximately the same length, although epibranchial 1(eb1) is small and slender compared with the more posterior epibranchials. Epibranchials 2 and 3 (eb2, eb3) have broad distal tips, and epibranchials 3 and 4 (eb3, eb4) are slightly bent, giving an overall angular appearance to the elements (Fig. 6).

The ventral gill arches (Fig. 7) comprise three ossified basibranchials (bb1–3), a posterior basibranchial copula (bb4), three hypobranchials (hb1–3), and five ceratobranchials (cb1–5). The basihyal and urohyal are closely associated with the ventral gill arch

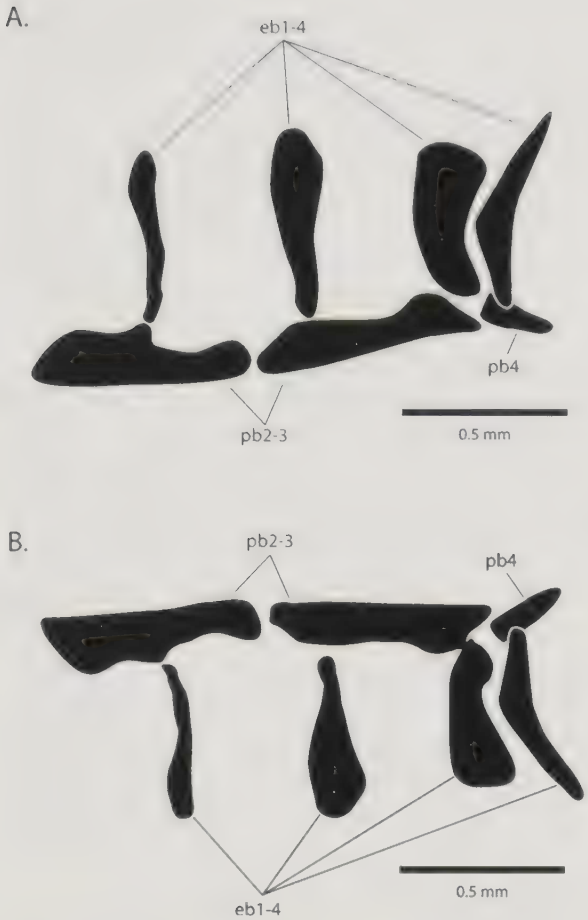

Figure 6. Dorsal gill arches of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Right side dorsal view and B, right side ventral view. VIMS 25002 (44.9 mm SL). Abbreviations: eb, epibranchial; pb, pharyngobranchial.

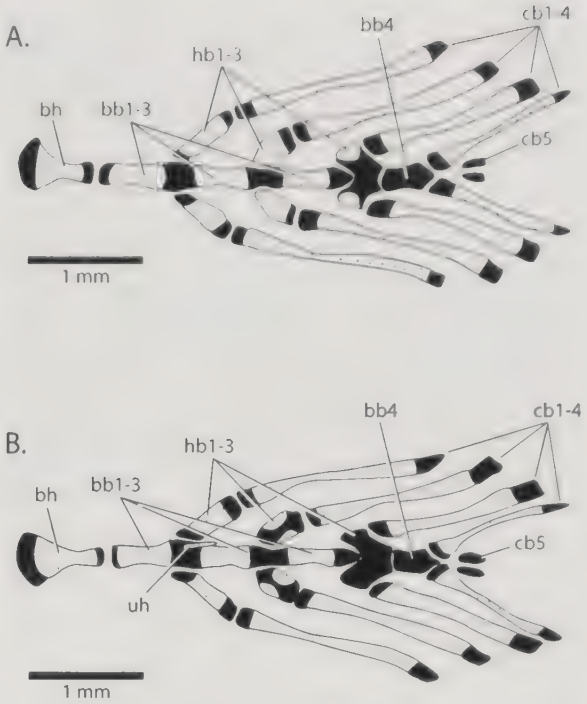

Figure 7. Ventral gill arches of *Parabrotula plagiophthalmus* Zugmayer, 1911. A, Dorsal and B, ventral views. VIMS 25002 (44.9 mm SL). Abbreviations: bb, basibranchial; bh, basihyal; cb, ceratobranchial; hb, hypobranchial; uh, urohyal.

skeleton and are described in the next paragraph. The three basibranchials are separated from one another by persistent cartilage of the anterior basibranchial copula (Fig. 7). Within the hypobranchial series, only the first hypobranchial is ossified completely, whereas the second is incomplete ventrally, and the third is only ossified on the dorsal surface of the hypobranchial cartilage. The left and right hypobranchial 3 (hb3) cartilages appear to be continuous across the midline, although this needs to be confirmed on additional material. The ceratobranchials are the longest elements of the ventral gill arches, and ceratobranchials 1 to 4 (cb1–4) are fairly uniform in shape and size (Fig. 7). Ceratobranchial 5 (cb5) is unique in that, even in the largest specimen examined, they are represented by small, irregularly shaped nodules of cartilage that are closely associated with the proximal tips of ceratobranchial 4 (Figs. 7, 8).

The basihyal is flared anteriorly, and its posterior cartilaginous tip is in close approximation with the anterior cartilaginous cap of the anterior basibranchial copula (Fig. 7). The urohyal is an exceptionally small sliver of bone positioned at the level of the cartilage between bb1 and bb2 (Fig. 7B).

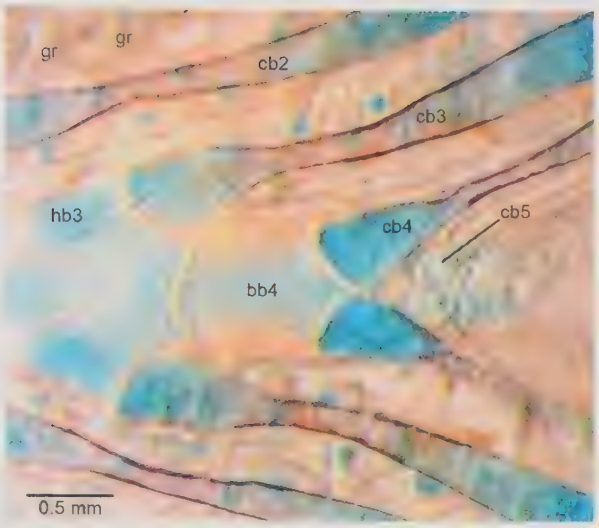

Figure 8. Close-up dorsal view of the posterior portion of the ventral gill arches of *Parabrotula plagiophthalmus* Zugmayer, 1911. VIMS 25002 (44.9 mm SL). Abbreviations: bb, basibranchial; cb, ceratobranchial; gr, gill raker; hb, hypobranchial.

Vertebral column

The vertebral column comprises 60–61 vertebrae in our three cleared and stained specimens and are well in the range for *P. plagiophthalmus* of 54–64 vertebrae described by Nielsen et al. (1990). Contrary to Nielsen (1968), however, we found the transition between abdominal and caudal vertebrae to be indistinct; therefore, our counts of abdominal and caudal vertebrae (made only on cleared and stained specimens) differ from those presented by Nielsen. In our specimens, we found the number of abdominal vertebrae to be 12 (VIMS 25002; Fig. 9), 17 (MCZ 14955), and 18 (MCZ 62267) and caudal vertebrae to be 49, 43, and 43, respectively. On the basis of radiographs, Nielsen (1968) reported 19–21 abdominal vertebrae and 35–39 caudal vertebrae. The haemal arches of the anteriormost caudal vertebrae are very similar in length to the posterior parapophyses of the abdominal region (e.g., Fig. 9B); therefore, in lateral view, it is virtually impossible to distinguish the vertebrae of the two regions. Only by observing them in frontal view is it possible to determine which of the vertebrae support a complete haemal arch (i.e., is a caudal vertebra). To complicate the situation further, we found the caudal centra 2 and 5 in one specimen (MCZ 14955) to not have a full haemal arch (i.e., the elongate haemal elements approach each other on these centra but do not fuse in the midline).

The vertebral centra of *P. plagiophthalmus* are remarkably uniform in size and shape along the entire length of the series, with the exception of the first vertebra, which is much shorter (Fig. 9A); near the posterior tip of the body, the vertebrae become slightly smaller (Fig. 9E). The neural arches and spines of all but the first two vertebrae are inclined posteriorly; those of the first two are oriented vertically or even slightly anteriorly (Fig. 9); the first vertebra of one specimen examined (MCZ 14955) does not have a neural arch. Weakly developed dorsal pre- and post-zygapophyses are best developed anteriorly on the body (Fig. 9).

Caudal fin and supports

The caudal fin of *P. plagiophthalmus* is formed by four to six fin rays (Nielsen et al. 1990; mode in that study was five); the three cleared and stained specimens examined here have six caudal fin rays (Fig. 9E). The compound preural 1 and ural centrum (i.e., the terminal vertebra) is small and posteriorly pointed. This vertebra supports dorsally a single epural and ventrally an autogenous parhypural; these two elements are nearly mirror images of each other, and each supports a single caudal fin ray (Figs. 9E, 10). There is a single, nearly triangular hypural element (Figs. 9E, 10), the proximal portion of which is ossified by a thin layer of bone. Four rays are associated with the hypural element in our specimens, all of

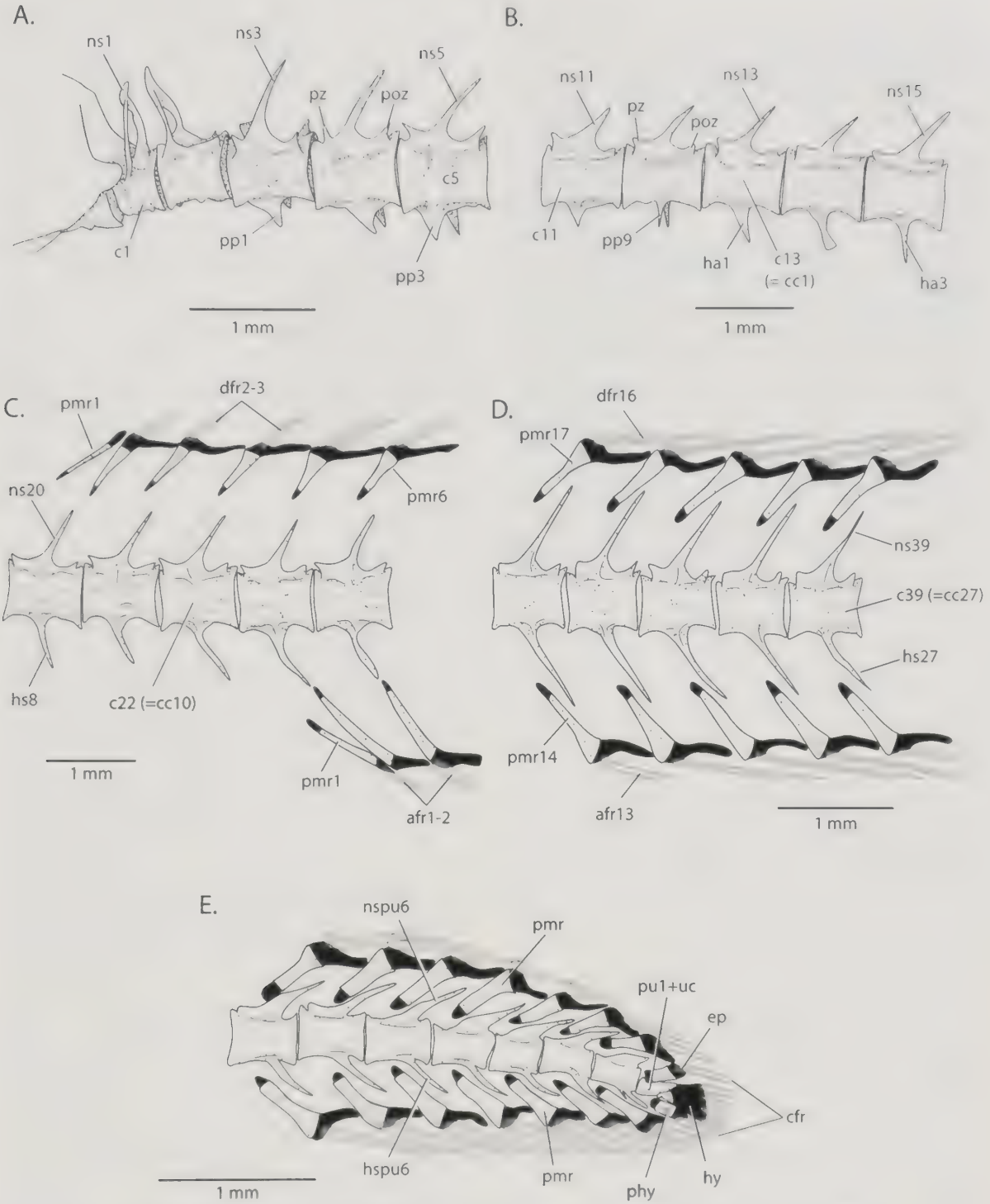

Figure 9. Vertebral column and median fins of *Parabrotula plagiophthalmus* Zugmayer, 1911. A. Anterior abdominal vertebrae. B. Transition between the abdominal and caudal regions. C. Caudal vertebrae at the anterior insertion of the dorsal and anal fins. D. Middle of the caudal region. E. Posteriormost portion of the caudal region. VIMS 25002 (44.9 mm SL). Abbreviations: afr, anal fin ray; c, centrum; cc, caudal centrum; cfr, caudal fin ray; dfr, dorsal fin ray; ep, epural; ha, haemal arch; hs, haemal spine; hspu, haemal spine of preural centrum; hy, hypural; ns, neural spine; nspu, neural spine of preural centrum; phy, parhypural; pmr, proximal middle radial; poz, postzygapophysis; pp, parapophysis; pu1+uc, compound preural centrum 1 and ural centrum; pz, prezygapophysis.

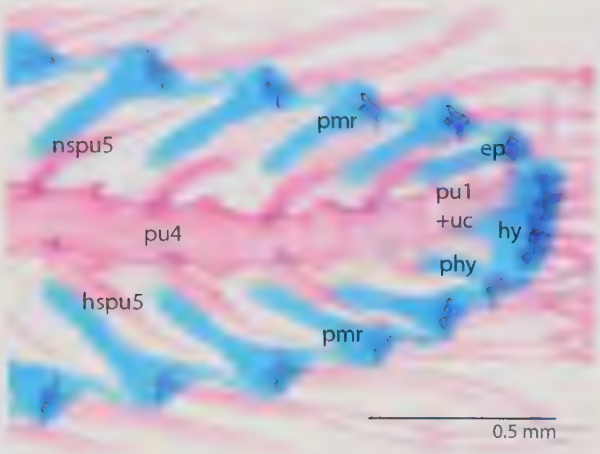

Figure 10. Caudal fin skeleton of *Parabrotula plagiophthalmus* Zugmayer, 1911. MCZ 62667 (41.6 mm SL). Abbreviations: ep, epural; hspu, haemal spine of preural centrum; hy, hypural; nspu, neural spine of preural centrum; phy, parhypural; pmr, proximal middle radial; pu, preural centrum; pu1+uc, compound preural centrum 1 and ural centrum.

which embrace the posterior cartilaginous portion of the element (Figs. 9E, 10).

Dorsal and anal fins and supports

The pterygiophores of the dorsal and anal fins of *P. plagiophthalmus* are mirror images of one another (Figs. 9C–E). The proximal-middle radials have a broad distal component and a thin tapering process that nearly reaches the anterior margin of the next posterior proximal-middle radial. The broad proximal portion of the proximal-middle radial is ossified by a thin collar of perichondral bone. There are no distal radials in the dorsal fin. In the anal fin of VIMS 25002 we found small distal radials in the pterygiophores associated with fin rays 4 (i.e., serially associated with the fifth pterygiophore), 7, 8, 10–14, and 16–20. These range from moderately well developed to just a few cartilage cells.

There are 37–43 (mode 40) dorsal fin rays and 34–39 (mode 36) anal fin rays in *P. plagiophthalmus* (Nielsen et al. 1990); we counted 43 dorsal fin rays and 38 anal fin rays in the two cleared and stained specimens that could be clearly counted (VIMS 25002; MCZ 62267). The fin rays are supernumerary in relation to the pterygiophores, such that the fin ray that is serially associated with a particular pterygiophore is functionally associated with the next posterior pterygiophore, articulating with the broad, mid portion of its proximal-middle radial.

Pectoral girdle and fin

The cleithrum is the largest and most heavily ossified element of the pectoral girdle in *P. plagiophthalmus*. It is smoothly curved and is pointed on both its dorsal and ventral ends (Fig. 11). The ventral tip of the cleithrum only weakly contacts its antimere. Both supracleithrum and posttemporal are represented by only small, slender bones (Fig. 11). The supracleithrum is oriented almost vertically, with a posteriorly curved portion ventrally, that contacts the lateral surface of the cleithrum. The posttemporal is a simple rod of bone (i.e., no ventral limb) and contacts the epioccipital.

The only ossification of the scapulocoracoid cartilage is the coracoid, which is a small collar of bone around the posteroventral extension of the cartilage (Fig. 11). Dorsally, a scapular foramen is partially obscured in lateral view (Fig. 11). The proximal pectoral radials of *P. plagiophthalmus* are represented by a single large cartilaginous plate. VIMS 25002 has a small opening in the ventral third of the cartilaginous plate (Fig. 11), but this plate was never observed to be divided into separate radial elements. There are no ossifications of the proximal radials, and there are no distal radials of any form in *P. plagiophthalmus*.

Nielsen (1968) reports six to eight pectoral fin rays, with almost all specimens examined having seven; we counted seven in our specimens. The pectoral fin rays are simple

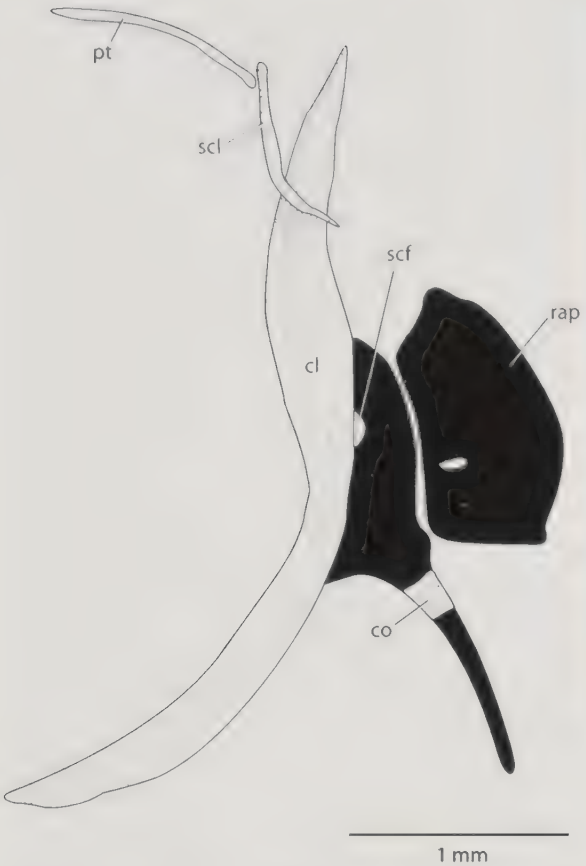

Figure 11. Pectoral girdle of *Parabrotula plagiophthalmus* Zugmayer, 1911. VIMS 25002 (44.9 mm SL). Abbreviations: cl, cleithrum; co, coracoid; pt, posttemporal; rap, radial plate; scf, scapular foramen; scl, supracleithrum.

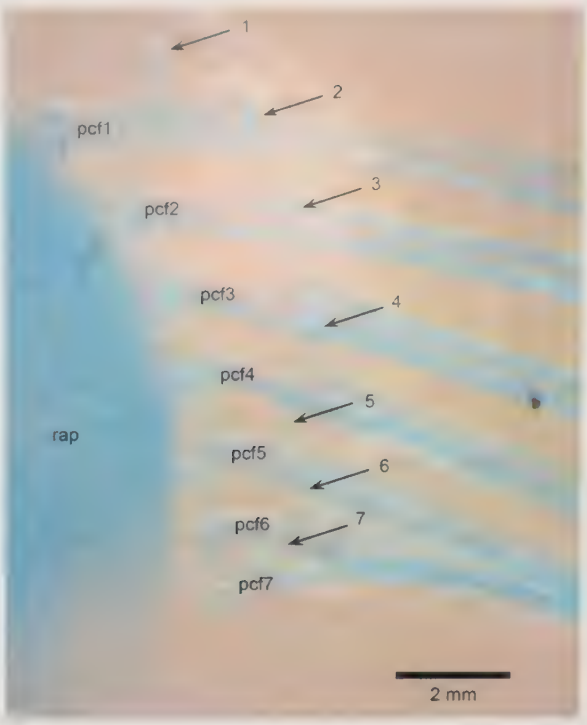

Figure 12. Close-up image of the pectoral fin base of *Parabrotula plagiophthalmus* Zugmayer, 1911. VIMS 25002 (44.9 mm SL). Abbreviations: pcf, pectoral fin ray; rap, radial plate. Numbered arrows indicate the dorsally directed hooks found at the base of each pectoral fin ray.

in form, with the exception of very pronounced, dorsally directed hooks at their proximal end (Fig. 12).

Pelvic girdle and fin

No pelvic fin rays or bones of the pelvic girdle are present in *P. plagiophthalmus*.

Scales and skin

There are no scales present in *P. plagiophthalmus*. As described by Nielsen (1968), the skin of *P. plagiophthalmus* appears to be loose on the body. Superficial neuromasts ("papillae" of Nielsen 1968) are along the trunk and on the head (i.e., forming the trunk, preoperculomandibular, supraorbital, and infraorbital lateral lines (Nielsen, 1968; EJH, personal observation).

DISCUSSION

When the genus *Parabrotula* was originally described, Zugmayer (1911a, b) assigned it to the family Brotulidae. At the time, Brotulidae included genera such as *Brotula* and *Dinematichthys* and several "aberrant" deep-sea taxa (e.g., *Aphyonus* and *Acanthonotus*; Regan, 1912). Nielsen (1968) reviewed the anatomy of *Parabrotula*, demonstrated its affinity to *Leucobrotula*, and concluded that they were, together, best regarded as a subfamily of Zoarcidae (Parabrotulinae) on the basis of a shared pattern of pterygio-

phore insertion between neural and heamal spines (1:1), among other characters (Anderson, 1994); this criterion was later used at the family level (Nielsen, 1973, 1986; Anderson, 1986), with affinities to zoarcoid fishes (e.g., considered near or within Zoarciformes). Nielsen et al. (1990) also regarded the two genera as close relatives of the Zoarcidae, as a family of the Zoarcoidei. Anderson (1994) soundly rejected this hypothesis, considering the two genera to be more closely related to Ophidiiformes (as he had proposed earlier; Anderson, 1984). We follow Anderson (1994) and regard *Parabrotula* (and by extension, Parabrotulidae) as morphologically similar to other Ophidiiformes.

Campbell et al. (2017) recently revisited the phylogenetic position of Parabrotulidae on the basis of 10 loci (16S, ENC1, FICD, KIAA1239, MYH6, PANX2, PTCHD4, RAG1, RHOD, and RIPK4). The results of this analysis, which included broad taxonomic sampling of both zoarcoid and ophidiiform taxa, suggest that Parabrotulidae (represented only by *P. tanseimaru*) is deeply nested within Bythitidae. In their analysis, *P. tanseimaru* was recovered as sister to *Bidenichthys capensis* with 90% bootstrap support. It is interesting to note that *Bidenichthys* is unique among Bythitidae analyzed by Møller et al. (2016) to have a free caudal fin (versus a caudal fin continuous with the dorsal and anal fins). The caudal fin rays of *Parabrotula* are continuous with those of the dorsal and anal fins (e.g., Fig. 1B; Nielsen, 1968: fig. 1). Zugmayer (1911b, plate 6, fig. 5, reproduced here as Fig. 1A) incorrectly showed the dorsal and anal fins in contact with the caudal fin, but the caudal fin is shown to be distinct.

With their description of the putatively basal bythitoid ophidiiform †*Pastorius methanyi*, Carnevale and Johnson (2015) summarized potential synapomorphies of the subgroups of Ophidiiformes. On the basis of that discussion, the morphology of *Parabrotula* is consistent with an ophidiiform (i.e., possessing an enlarged exoccipital, with the supraoccipital excluded from the posterior margin of the braincase). In addition to the soft tissue characters of the male reproductive system (i.e., intromittent organ), the osteology of *Parabrotula* is consistent with characters of bythitoids (e.g., absence of an interarcual cartilage). Details of the intromittent organ (continuous with the sheath versus separate from the sheath) suggest that *Parabrotula* is allied with Bythitidae, as opposed to Dinematicthyidae (e.g., compare Nielsen, 1968: fig. 4 [*Parabrotula*] with Møller et al., 2016, fig. 1B–D [Bythitidae] versus Møller et al., 2016, fig. 1A [*Dinematichthys*]). Furthermore, the presence of only six caudal fin rays is consistent with the condition in bythitine bythitids (versus brosmophycine bythitids) and "aphyonids" (Carnevale and Johnson, 2015). Similarly, the confluence of the dorsal, caudal, and anal fins is suggestive that *Parabrotula* is not a brosmophycine bythitid (the separation of these fins is unique to brosmophycines among Ophidiiformes; Carnevale and Johnson, 2015). Of the 11 traits cited by Nielsen (1969) as diagnostic of Aphyonidae, *Parabrotula* is similar in being viviparous and having a long, slender body; loose skin with no scales; confluent dorsal, anal, and caudal fins; absence of free spines on the gill cover; an undeveloped swim bladder (Nielsen, 1968); and anteriormost neural spines similar in length to the more posterior ones. However, these characters are a mix of plesiomorphic and apomorphic characters for bythitoid fishes, and further study is necessary to determine their phylogenetic distribution and the relationships within Bythitoidei.

Parabrotula has been regarded as morphologically truncated (Campbell et al., 2017). Indeed, a few aspects of the skeleton

resemble early life history stages of other fishes (e.g., the pectoral fin radial plate, which never differentiates into separate elements). The most striking instance of reduction per se that we observed is in the gill arch skeleton (extreme reduction of the fifth ceratobranchial; discussed below), with all other elements present. However, we have been impressed that although small and thinly developed, the skeleton of *Parabrotula* is relatively well formed and complete despite the small body size of the taxon, unlike the example noted by Campbell et al. (2017; e.g., the cypriniform *Paedocypris*) and other so-called "reduced" taxa. This is not to say that there are no elements missing (e.g., infraorbital bones, pelvic bones and fins, and scales) but rather that these elements are frequently missing in other radiations that are not considered overtly "morphologically reduced."

Two aspects of our results are worthy of further discussion. First, within *P. plagiophthalmus* we found the pars autopalatina and posterior portion of palatoquadrate to be discontinuous (i.e., reduction of the processus pterygoideus; no cartilage between the two regions), with only a very thin ectopterygoid bridging this gap. As far as we know, a reduced processus pterygoideus is only known for the tetraodontiform families Balistidae and Monacanthidae (Konstantinidis and Johnson, 2012).

One of the most striking aspects of the osteology of *P. plagiophthalmus* is the reduction of the fifth ceratobranchial. Nielsen (1968) described both *Parabrotula* and *Leucobrotula* as having "3½ gill arches." As shown earlier in this text, however, the gill arches of *P. plagiophthalmus* are complete, in that all elements are present. However, the ceratobranchials 5 are reduced to the point that they could be easily overlooked. Past reports of incomplete or absent gill arches may be based on ontogenetic variation, because most other specimens available are smaller than those examined here. Other bythitoid taxa examined here (e.g., *Dinematichthys*) have complete ventral gill arches (i.e., cb5 is present and large). To our knowledge, the absence of a cb5 is found only in muraenid eels (Johnson, 2019) and the aulopiform *Gigantura indica* (Konstantinidis and Johnson, 2016), although the reduction in these taxa is clearly a different condition (i.e., more extreme reduction of more anterior arches, absence of basibranchials, and, at least in *Gigantura*, loss of all ceratobranchials except cb4). Further study of the morphology of Bythitidae, particularly putatively close relatives of *Parabrotula* (e.g., *Bidenichthys*) is warranted.

ACKNOWLEDGMENTS

For access to specimens in their care, we thank K. Hartel (MCZ), K. Maslenikov and L. Tornabene (UW), and for donation of the specimens to VIMS, we thank T. Sutton (Nova Southeastern). For curatorial assistance, we thank S. Huber (VIMS). J. Nielsen graciously supplied electronic copies of his papers. All images of MCZ specimens are © President and Fellows of Harvard College. Drafting of this manuscript benefited from the support of the Winter 2020 William & Mary Faculty Writers' Retreat, sponsored by the Provost's Office and William & Mary Libraries. This is contribution 3946 of the Virginia Institute of Marine Science, William & Mary.

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